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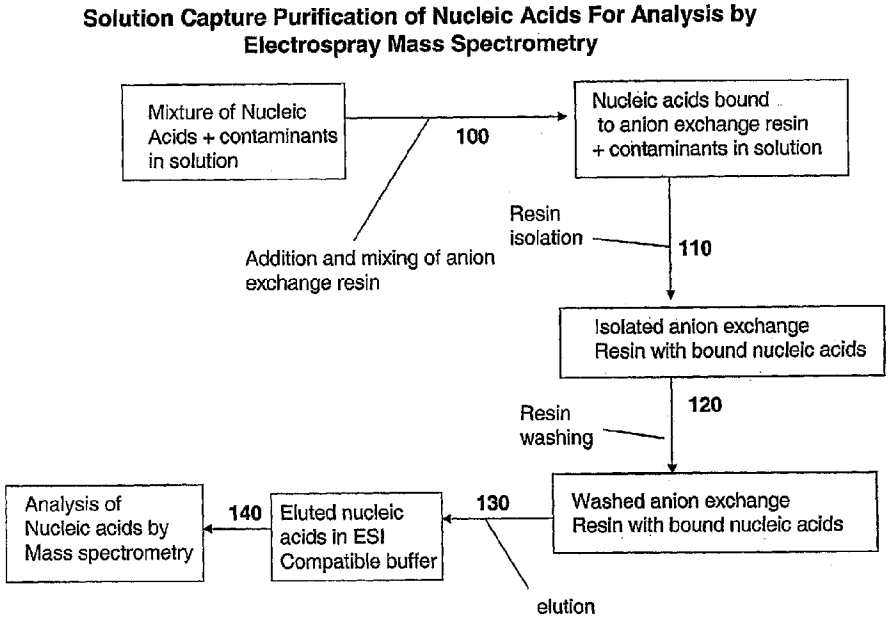

(57) **Abstract:** The present invention provides methods for rapid solution capture purification of nucleic acids for subsequent analysis by electrospray mass spectrometry which is efficient and cost-effective relative to existing methods. The present invention also provides for kits useful for practicing rapid solution capture of nucleic acids so that purified samples are in condition for analysis by electrospray mass spectrometry.

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METHODS FOR RAPID PURIFICATION OF NUCLEIC ACIDS FOR SUBSEQUENT ANALYSIS BY MASS SPECTROMETRY BY SOLUTION CAPTURE

FIELD OF THE INVENTION

The present invention relates generally to the field of analysis of nucleic acids by mass spectrometry and provides methods and kits useful for this purpose.

BACKGROUND OF THE INVENTION

Electrospray ionization mass spectrometry (ESI-MS) has become an important technique for the analysis of biopolymers. The multiple charging phenomenon allows fast, accurate and precise molecular mass measurement, identification of modifications and more detailed structural studies for very high-mass biopolymers.

Amplification of specific DNA sequences utilizing the polymerase chain reaction has widespread applications in many scientific disciplines, including microbiology, medical research, forensic analysis, and clinical diagnostics. Most often, PCR products are "sized" using traditional biochemical techniques such as standard gel electrophoresis using either intercalating dyes or fluorescently labeled primers. The Taqman™ assay, which is widely used in a number of PCR-based diagnostic kits, confirms the presence (or absence) of a specific PCR product but provides no direct readout on the size of the amplicon. So-called "real-time" PCR devices, which measure the laser-induced fluorescence of the PCR product during the amplification cycles, are used to quantify the amplification of DNA from a given DNA template and primer set. These methods have limited utility for relatively small amplicons (less than 150 base pairs), owing to the proportionately high fluorescence background, and do not provide any information with respect to amplicon heterogeneity or exact length.

Compared to these more traditional methods, mass spectrometry has several potential advantages as a platform on which to characterize PCR products, including speed, sensitivity, and mass accuracy. Because the exact mass of each of the bases which comprise DNA is known with great accuracy, a high-precision mass measurement obtained via mass spectrometry can be used to derive a base composition within the experimentally obtained mass measurement uncertainty (J. Aaserud, Z. Guan, D.P. Little and F.W. McLafferty, *Int. J. Mass Spectrom. Ion Processes*, **1997**, *167/168*, 705–712. and D.C. Muddiman, G.A. Anderson, S.A. Hofstadler and R.D. Smith, *Anal. Chem.* **1997**, *69*, 1543–1549). Methods for rapid identification of unknown bioagents using a combination of nucleic acid amplification and determination of base

composition of informative amplicons by molecular mass analysis are disclosed and claimed in published U.S. Patent applications 20030027135, 20030082539, 20030124556, 20030175696, 20030175695, 20030175697, and 20030190605 and U.S. patent application Serial Nos. 10/326,047, 10/660,997, 10/660,122 and 10/660,996, all of which are commonly owned and incorporated herein by reference in entirety.

Both MALDI (matrix assisted, laser desorption ionization) and electrospray (ESI) mass spectrometry have been employed to ionize PCR products for subsequent mass spectrometric detection. While MALDI is widely used to analyze short (20-mer or smaller) oligonucleotides, applications to amplicons in excess of 100 bp are less common. ESI is one of the most widely used ionization techniques for large biological molecules owing to the inherent "softness" of the ionization process, which allows DNA in excess of 500 bp to be ionized without dissociation.

In ESI, large charged droplets are produced in the process of "pneumatic nebulization" where the analyte solution is forced through a needle at the end of which is applied a potential sufficient to disperse the emerging solution into a very fine spray of charged droplets all of which have the same polarity. The solvent evaporates, shrinking the droplet size and increasing the charge concentration at the droplet's surface. Eventually, at the Rayleigh limit, Coulombic repulsion overcomes the droplet's surface tension and the droplet explodes. This "Coulombic explosion" forms a series of smaller, lower charged droplets. The process of shrinking followed by explosion is repeated until individually charged analyte ions are formed. The charges are statistically distributed amongst the analyte's available charge sites, leading to the possible formation of multiply charged ions conditions. Increasing the rate of solvent evaporation, by introducing a drying gas flow counter current to the sprayed ions, increases the extent of multiple-charging. Decreasing the capillary diameter and lowering the analyte solution flow rate i.e. in nanospray ionization, will create ions with higher m/z ratios (i.e. it is a softer ionization technique) than those produced by "conventional" ESI and are of much more use in the field of bioanalysis.

Unfortunately, ESI requires relatively clean samples and is notoriously intolerable of cationic salts, detergents, and many buffering agents commonly used in biochemical laboratories.

The buffer system commonly employed in the polymerase chain reaction includes electrospray incompatible reagents such as 50 mM KCl, 2 mM $MgCl_2$, 10 mM Tris–HCl, and each of the four deoxynucleotide triphosphates (dNTPs) at 200 :M. Even the presence of relatively low concentrations of metal cations (less than 100 :M) can significantly reduce MS sensitivity for oligonucleotides as the signal for each molecular ion is spread out over multiple salt adducts. Thus, in addition to removing detergents and dNTPs, effective ESI-MS of PCR

products requires that the salt concentration be reduced by more than a factor of 1000 prior to analysis.

Ethanol precipitation has been used to desalt PCR products for subsequent MS analysis as short oligonucleotides and salts are removed while the sample is concentrated (M.T. Krahmer, Y.A. Johnson, J.J. Walters, K.F. Fox, A. Fox and M. Nagpal, Electrospray Anal. Chem. 1999, *71*, 2893–2900; T. Tsuneyoshi, K. Ishikawa, Y. Koga, Y. Naito, S. Baba, H. Terunuma, R. Arakawa and D.J. Prockop *Rapid Commun. Mass Spectrom.* **1997**, *11,* 719–722; and D.C. Muddiman, D.S. Wunschel, C.L. Liu, L. Pasatolic, K.F. Fox, A. Fox, G.A. Anderson and R.D. Smith *Anal. Chem.* **1996**, *68,* 3705–3712). In this method, the PCR product can be precipitated from concentrated ammonium acetate solutions, either overnight at 5 °C or over the course of 10–15 min with cold (-20 °C) ethanol. Unfortunately, a precipitation step alone is generally insufficient to obtain PCR products which are adequately desalted to obtain high-quality ESI spectra; consequently, precipitation is generally followed by a dialysis step to further desalt the sample (D.C. Muddiman, D.S. Wunschel, C.L. Liu, L. Pasatolic, K.F. Fox, A. Fox, G.A. Anderson and R.D. Smith *Anal. Chem.* **1996**, *68,* 3705–3712). While several researchers have successfully employed these methods to characterize a number of PCR products, the route to applying these methods in a robust and fully automated high-throughput manner is not obvious.

Commercial DNA purification kits may also be used in conjunction with traditional desalting techniques such as microdialysis (S. Hahner, A. Schneider, A. Ingendoh and J. Mosner *Nucleic Acids Res.* **2000**, *28,* e82/i–e82/viii; and A.P. Null, L.T. George and D.C. Muddiman *J. Am. Soc. Mass Spectrom.* 2002, *13*, 338–344). Other purification techniques, such as gel electrophoresis followed by high-performance liquid chromatography or drop dialysis, or cation exchange using membranes or resins have also been used to obtain high-purity, desalted DNA for MS detection (L.M. Benson, S.-S. Juliane, P.D. Rodringues, T. Andy, L.J. Maher III and S. Naylor, In: *The 47th ASMS Conference on Mass Spectrometry and Allied Topics*, Dallas, Texas (1999); C.G. Huber and M.R. Buchmeiser *Anal. Chem.* 1998, *70*, 5288–5295; H. Oberacher, W. Parson, R. Muehlmann and C.G. Huber *Anal. Chem.* **2001**, *73,* 5109–5115; and C.J. Sciacchitano *J. Liq. Chromatogr. Relat. Technol.* **1996**, *19,* 2165–2178). Unfortunately, as with the techniques described above, the path toward a rapid and fully automated high-throughput implementation is not obvious.

Jiang and Hofstadler have developed and reported a single protocol for the purification and desalting of PCR products which employs commercially available pipette tips packed with anion exchange resin (Y. Jiang and S. A. Hofstadler *Anal. Biochem.* **2003**, *316*, 50-57). This protocol yields an ESI-MS-compatible sample and requires only 10 :l of crude PCR product.

However, the method is cost-prohibitive when applied to high volume and high throughput processes such as the methods cited above for identification of unknown bioagents. Retail costs of using the commercially-obtained ZipTip™ AX (Millipore Corp. Bedford, MA) are estimated at $1.77 per plate well.

There remains a need for a method of purification of nucleic acids for mass spectrometry which is rapid, efficient and non-cost prohibitive. The present invention satisfies this need.

Solution capture of nucleic acids such as those obtained from amplification reactions has enabled a rapid, cost-effective method of extracting and purifying these analytes for subsequent analysis by mass spectrometry. Since the nucleic acids and the anion exchange media are in solution, efficient capture of the nucleic acids is accomplished by vortexing, or other mixing methods. This has eliminated the need to pack the media in a column format which would require multiple passes of the nucleic acid solution over it to achieve high levels of recovery of nucleic acids. While longer columns require fewer passes, significant backpressure becomes a problem. The process of packing an anion exchange resin in a column or pipette tip format increases the cost associated with the procedure accordingly. Thus the use of solution capture for purification of PCR products for analysis by mass spectrometry has substantially reduced the cost associated with sample preparation by eliminating the need to pack, equilibrate, and test a column. The retail cost of the current procedure using a pipette tip packed with anion exchange resin exemplified by ZipTip™ AX (Millipore, Bedford, MA) is approximately $1.77 per pipette tip (for each sample). The estimated cost of solution capture of PCR products is $0.10 per sample and takes into account the combination of anion exchange resin and filter plate. Furthermore, the time required for solution capture purification of PCR products is approximately 10 minutes per 96 well plate in contrast to the previous method which employs the ZipTip™ AX pipette tips and requires approximately 20 minutes.

SUMMARY OF THE INVENTION

The present invention is directed to, *inter alia,* solution capture methods of purifying a solution comprising one or more nucleic acids for subsequent analysis by electrospray mass spectrometry, or any other analysis, by adding an anion exchange resin to the solution and mixing to yield a suspension of the anion exchange resin in the solution wherein the nucleic acid binds to the anion exchange resin, isolating the anion exchange resin from the solution, washing the anion exchange resin to remove one or more contaminants with one or more wash buffers while retaining bound nucleic acid, eluting the nucleic acid, from the ion exchange resin with an elution buffer, and optionally, analyzing the nucleic acids by electrospray mass spectrometry.

The anion exchange resin may have a strong anion exchange functional group such as a quaternary amine or a weak anion exchange functional group such as, for example, polyethyleneimine, charged aromatic amine, diethylaminomethyl, or diethylaminoethyl. Such weak anion exchange resins comprise functional groups with pK_a values of 9.0 or greater.

The present invention is further directed to kits for purification of nucleic acids comprising a filter plate comprising a plurality of wells or a tube rack comprising a plurality of tubes, an anion exchange resin, at least one anion exchange wash buffer and an ESI-MS-compatible elution buffer.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a process diagram outlining the steps of the present invention beginning with the addition and mixing of anion exchange resin into the sample of nucleic acids (**100**). The resin is then isolated from the solution (**110**) and washed with an appropriate wash buffer to remove contaminants from the resin (**120**) after which, the nucleic acids are eluted from the resin by an electrospray ionization (ESI)-compatible elution buffer, which makes possible the final step of analysis of the nucleic acids by ESI-mass spectrometry (**140**).

Figure 2 is a comparison of ESI-MS spectra for purified PCR products obtained by purification with ZipTips™ (top panel) and by the solution capture purification method of the present invention. The comparison indicates that purification by the solution capture method is equally effective as the previously validated method which employs ZipTips™.

DESCRIPTION OF EMBODIMENTS

One embodiment of the method of solution capture purification of nucleic acids for analysis by mass spectrometry, for example, is outlined in Figure 1. The methods described herein can be used for other types of analysis, in addition to mass spectrometry as known to those skilled in the art. The methods comprise the following steps: Addition and mixing of an anion exchange resin into a solution of nucleic acids (**100**), isolating the anion exchange resin from the solution (**110**), washing the anion exchange resin to remove contaminants (**120**), eluting the nucleic acids, (free of contaminants) from the anion exchange resin (**130**), and, optionally, analyzing the nucleic acid by ESI mass spectrometry.

In some embodiments, a strong cation exchange functional group, such as a quaternary amine for example, is employed as the functional group of the anion exchange resin. Additional strong anion exchange functional groups are known to those skilled in the art.

In other embodiments, a weak anion exchange functional group is a suitable anion exchange functional group, such as polyethyleneimine, charged aromatic amine, diethylaminomethyl, or diethylaminoethyl, for example, are employed as the functional group of the anion exchange resin. Such functional groups have pK_a values of 9.0 or greater. Commercial products of weak anion exchange resin include, but are not limited to; Baker PEI, Baker DEAM, Dionex ProPac™ WAX, Millipore PEI, Applied Biosystems Poros™ PI.

In some embodiments, the mixing of the anion exchange resin into the solution of nucleic acids is effected by repeated pipetting, vortexing, sonication, shaking, or any other method that results in suspension of the anion exchange resin in the solution containing the nucleic acids.

In some embodiments, dry anion exchange resin is added directly to the solution of nucleic acids or contained within a microtube or the well of a micro filter plate into which the solution of nucleic acids is added prior to mixing. In other embodiments, the anion exchange resin is pre-hydrated and added directly to the solution of nucleic acids or contained within a microtube or a well of a microfilter plate into which the solution of nucleic acids is added prior to mixing.

In some embodiments, the anion exchange resin which contains bound nucleic acids is isolated from the solution by filtration. Filtration can be effected, for example, using a filter plate in a 96- or 384-well format which enables high-throughput purification of multiple samples, or in any other container or plurality of containers equipped with a filter. Other well format plates can also be used. Membranes useful for filtration include but are not limited to those composed of the following materials: polytetrafluoroethylene (PTFE), polyvinyldifluoro (PVDF), polypropylene, polyethylene, glass fiber, polycarbonate and polysulfone. Filtering may by accomplished by vacuum, centrifugation, or positive pressure displacement with fluids or gases, or any other method that effects the isolation of the anion exchange resin from the solution. Methods of filtering are well known to those skilled in the art.

In some embodiments, the anion exchange resin comprises an anion exchange functional group which is linked to magnetic beads. Such an arrangement enables a simpler isolation step (**110**) by eliminating the need for centrifugation, vacuum or positive pressure displacement which would necessitate the removal of the plate or microtube tube from the liquid handler deck. Instead, a magnetic field can be activated to compress the magnetic bead resin so that liquid can be aspirated off by the liquid handler. Methods of using magnetic beads to effect isolation of biomolecules are well known to those skilled in the art.

In some embodiments, the anion exchange resin which contains bound nucleic acids is washed to remove one or more contaminants. Contaminants include, but are not limited to:

proteins such as reverse transcriptase and restriction enzymes, polymers, salts, buffer additives, or any of the various components of an amplification reaction such as polymerases nucleotide triphosphates or any combination thereof. Depending on the composition of the contaminants in the nucleic acid solution, more than one wash buffer may be useful for removal of contaminants. Washing of the anion exchange resin can be effected with aqueous solutions of ammonium acetate in the millimolar range from about 20 mM to about 500 mM NH_4OAc or with about 20 mM to about 500 mM NH_4HCO_3. Washing with about 10% to about 50% methanol, about 20% to about 50% methanol, or about 10% to about 30% methanol is useful as a final wash step. Methanol can be replaced by other suitable alcohols known to those skilled in the art.

In some embodiments, elution of nucleic acids from the anion exchange resin is accomplished using an ESI-compatible solution at alkaline pH of about pH 9 or greater such as an aqueous solution of about 2% to about 8% ammonium hydroxide or an aqueous solution of about 10 mM to about 50 mM, or 25mM piperidine, about 10 mM to about 50 mM, or 25mM imidazole and about 30% methanol or other suitable alcohol. As defined herein, an ESI-compatible solution is a solution which does not have a detrimental effect on the function of an electrospray (ESI) source.

As used herein, the term "about" means ± 10% of the term being modified. Thus, for example, "about" 10 mM means 9 to 11 mM.

In another embodiment, the present invention also provides kits for purification of nucleic acids by the solution capture method of the present invention. In some embodiments, the kit may comprise a sufficient quantity of anion exchange resin. In some embodiments, the anion exchange resin is a weak anion exchange resin such as one of the following commercially available weak anion exchange resins: Baker polyethyleneimine resin, Baker diethylaminomethyl resin, Dionex ProPac™ WAX, Millipore polyethyleneimine, and Applied Biosystems POROS™ PI.

In some embodiments, the kit may comprise a filter plate such as a 96- or 384-well filter plate or a microtube rack comprising a plurality of micro filter tubes.

In some embodiments, dry anion exchange resin is pre-loaded into the wells of a filter plate or microtube rack and can be either pre-hydrated or in the dry (powder) form.

The kit may also comprise a filter plate comprising a plurality of wells or a tube rack comprising a plurality of tubes, an anion exchange resin, at least one anion exchange wash buffer and an ESI-MS-compatible elution buffer.

In one embodiment, the kit may comprise a 96 or 384 well plate containing either pre-hydrated anion exchange resin or dry anion exchange resin, a second 96 or 384 well sample

mixing plate, a 96 or 384 well filter plate, a resin treatment buffer, one or more wash buffers, and an ESI-compatible elution buffer.

In one embodiment, the nucleic acid solution is a PCR product prepared for identification of an unknown bioagent and contained in an individual well of a 96 well sample plate on the deck of an automated liquid handler. The liquid handler is the cornerstone for many laboratory processes associated with drug discovery and high throughput screening. The dispensing and aspiration functions of liquid handlers are used to perform solvent/reagent additions, dilutions, plate replications consolidation, redistribution and other microplate-based tasks and typically use disposable pipette tips for transferring liquids. Programming of liquid handlers to perform the various liquid handling tasks of this embodiment is well within the capabilities of one with ordinary skill in the art without undue experimentation.

The liquid handler is programmed to transfer and mix a predetermined volume of a suspension of anion exchange resin into the well containing the PCR product. The resin suspension can be contained in a resin source container such a 96 well plate and transferred to the PCR product plate by the liquid handler. Mixing is performed by the liquid handler via repeated dispensation and aspiration of the PCR-resin mixture and binding of nucleic acids to the resin occurs at this stage. Next, the liquid handler transfers the PCR product-resin mixture from the 96 well plate to a 96 or 384 well filter plate. At this stage, the filter plate can be removed from the liquid handler deck and the resin can be isolated from the solution by centrifugation or positive pressure displacement before returning the filter plate to the liquid handler deck.

The resin containing bound nucleic acids is then washed one or more times with an appropriate wash solution such as about 100 mM NH_4HCO_3 with the liquid handler pipetting the wash solution into the filter plate, followed by centrifugation, vacuum, or positive pressure displacement followed by one or more washes with about 20% to about 50% methanol before returning the filter plate containing the resin and bound nucleic acids to the liquid handler deck.

Finally, the nucleic acids are eluted from the resin with an ESI compatible elution buffer such as an aqueous solution of about 25mM piperidine, about 25mM imidazole and about 50% methanol. This ESI compatible buffer may also optionally contain an internal standard used to calibrate the ESI mass spectrometer during the subsequent ESI mass spectrometry analysis.

In order that the invention disclosed herein may be more efficiently understood, examples are provided below. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the invention in any manner. Throughout these examples, molecular cloning reactions and other standard recombinant DNA techniques were carried out according to methods described in Maniatis et al., Molecular Cloning – A Laboratory Manual,

2nd ed. Cold Spring Harbor Press (1989), using commercially available reagents except where otherwise noted.

EXAMPLES

Example 1: Nucleic Acid Isolation and PCR

In one embodiment, nucleic acid is isolated from the organisms and amplified by PCR using standard methods prior to BCS determination by mass spectrometry. Nucleic acid is isolated, for example, by detergent lysis of bacterial cells, centrifugation and ethanol precipitation. Nucleic acid isolation methods are described in, for example, *Current Protocols in Molecular Biology* (Ausubel et al.) and *Molecular Cloning; A Laboratory Manual* (Sambrook *et al.*). The nucleic acid is then amplified using standard methodology, such as PCR, with primers which bind to conserved regions of the nucleic acid which contain an intervening variable sequence as described below.

General Genomic DNA Sample Prep Protocol: Raw samples are filtered using Supor-200 0.2 μM membrane syringe filters (VWR International). Samples are transferred to 1.5 ml eppendorf tubes pre-filled with 0.45 g of 0.7 mm Zirconia beads followed by the addition of 350 μl of ATL buffer (Qiagen, Valencia, CA). The samples are subjected to bead beating for 10 minutes at a frequency of 19 l/s in a Retsch Vibration Mill (Retsch). After centrifugation, samples are transferred to an S-block plate (Qiagen) and DNA isolation is completed with a BioRobot 8000 nucleic acid isolation robot (Qiagen).

Swab Sample Protocol: Allegiance S/P brand culture swabs and collection/transport system are used to collect samples. After drying, swabs are placed in 17x100 mm culture tubes (VWR International) and the genomic nucleic acid isolation is carried out automatically with a Qiagen Mdx robot and the Qiagen QIAamp DNA Blood BioRobot Mdx genomic preparation kit (Qiagen, Valencia, CA).

Example 2: Mass spectrometry

The mass spectrometer used is a Bruker Daltonics (Billerica, MA) Apex II 70e electrospray ionization Fourier transform ion cyclotron resonance mass spectrometer (ESI-FTICR-MS) that employs an actively shielded 7 Tesla superconducting magnet. All aspects of pulse sequence control and data acquisition were performed on a 1.1 GHz Pentium II data station running Bruker's Xmass software. 20 μL sample aliquots were extracted directly from 96-well microtiter plates using a CTC HTS PAL autosampler (LEAP Technologies, Carrboro, NC)

triggered by the data station. Samples were injected directly into the ESI source at a flow rate of 75 μL/hr. Ions were formed via electrospray ionization in a modified Analytica (Branford, CT) source employing an off axis, grounded electrospray probe positioned ca. 1.5 cm from the metalized terminus of a glass desolvation capillary. The atmospheric pressure end of the glass capillary is biased at 6000 V relative to the ESI needle during data acquisition. A counter-current flow of dry N_2/O_2 was employed to assist in the desolvation process. Ions were accumulated in an external ion reservoir comprised of an rf-only hexapole, a skimmer cone, and an auxiliary gate electrode, prior to injection into the trapped ion cell where they were mass analyzed.

Spectral acquisition was performed in the continuous duty cycle mode whereby ions were accumulated in the hexapole ion reservoir simultaneously with ion detection in the trapped ion cell. Following a 1.2 ms transfer event, in which ions were transferred to the trapped ion cell, the ions were subjected to a 1.6 ms chirp excitation corresponding to 8000 – 500 m/z. Data was acquired over an m/z range of 500 – 5000 (1M data points over a 225K Hz bandwidth). Each spectrum was the result of co-adding 32 transients. Transients were zero-filled once prior to the magnitude mode Fourier transform and post calibration using the internal mass standard. The ICR-2LS software package (G. A. Anderson, J. E. Bruce (Pacific Northwest National Laboratory, Richland, WA, 1995) was used to deconvolute the mass spectra and calculate the mass of the monoisotopic species using an "averaging" fitting routine (M. W. Senko, S. C. Beu, F. W. McLafferty, *J. Am. Soc. Mass Spectrom.* **1995**, *6*, 229) modified for DNA. Using this approach, monoisotopic molecular weights were calculated.

Example 3: Procedure for Semi-automated Purification of a PCR mixture using Commercially Available ZipTips™

For pre-treatment of ZipTips™ AX (Millipore Corp. Bedford, MA), the following steps were programmed to be performed by an Evolution™ P3 liquid handler (Perkin Elmer) with fluids being drawn from stock solutions in individual wells of a 96-well plate (Marshall Bioscience): loading of a rack of ZipTips™ AX; washing of ZipTips™ AX with 15 :l of 10% NH_4OH/50% methanol; washing of ZipTips™ AX with 15 :l of water 8 times; washing of ZipTips™ AX with 15 :l of 100 mM NH_4OAc.

For purification of a PCR mixture, 20 :l of crude PCR product was transferred to individual wells of a MJ Research plate using a BioHit™ multichannel pipette. Individual wells of a 96-well plate were filled with 300 :l of 40 mM NH_4HCO_3. Individual wells of a 96-well plate were filled with 300 :l of 20% methanol. An MJ research plate was filled with 10 :l of 4% NH_4OH. Two reservoirs were filled with deionized water. All plates and reservoirs were placed

on the deck of the Evolution™ P3 (EP3) pipetting station in pre-arranged order. The following steps were programmed to be performed by an Evolution™ P3 pipetting station: aspiration of 20 :l of air into the EP3 P50 head; loading of a pre-treated rack of ZipTips™ AX into the EP3 P50 head; dispensation of the 20 :l NH_4HCO_3 from the ZipTips™ AX; loading of the PCR product into the ZipTips™ AX by aspiration/dispensation of the PCR solution 18 times; washing of the ZipTips™ AX containing bound nucleic acids with 15 :l of 40 mM NH_4HCO_3 8 times; washing of the ZipTips™ AX containing bound nucleic acids with 15 :l of 20% methanol 24 times; elution of the purified nucleic acids from the ZipTips™ AX by aspiration/dispensation with 15 :l of 4% NH_4OH 18 times. For final preparation for analysis by ESI-MS, each sample was diluted 1:1 by volume with 70% methanol containing 50 mM piperidine and 50 mM imidazole.

Example 4: Procedure for Semi-automated Purification of a PCR mixture with Solution Capture

For pre-treatment of ProPac™ WAX weak anion exchange resin, the following steps were performed in bulk: sequential washing three times (10:1 volume ratio of buffer to resin) with each of the following solutions: (1) 1.0 M formic acid/50% methanol (2) 20% methanol (3) 10% NH_4OH (4) 20% methanol (5) 40 mM NH_4HCO_3 (6) 100 mM NH_4OAc. The resin is stored in 20 mM NH_4OAc/50% methanol at 4°C.

Corning 384-well glass fiber filter plates were pre-treated with two rinses of 250 :l NH_4OH and two rinses of 100 :l NH_4HCO_3.

For binding of the PCR product nucleic acids to the resin, the following steps were programmed to be performed by the Evolution™ P3 liquid handler: addition of 0.05 to 10 :l of pre-treated ProPac™ WAX weak anion exchange resin (30 :l of a 1:60 dilution) to a 50 :l PCR reaction mixture (80 :l total volume) in a 96-well plate; mixing of the solution by aspiration/dispensation for 2.5 minutes; and transfer of the solution to a pre-treated Corning 384-well glass fiber filter plate. This step was followed by centrifugation to remove liquid from the resin and is performed manually, or under the control of a robotic arm.

The resin containing nucleic acids was then washed by rinsing three times with 200 :l of 100 mM NH_4OAc, 200 :l of 40 mM NH_4HCO_3 with removal of buffer by centrifugation for about 15 seconds followed by rinsing three times with 20% methanol for about 15 seconds. The final rinse was followed by an extended centrifugation step (1-2 minutes).

Elution of the nucleic acids from the resin was accomplished by addition of 40 :l elution/electrospray buffer (25 mM piperidine/25 mM imidazole/35% methanol and 50 nM of an

internal standard oligonucleotide for calibration of mass spectrometry signals) followed by elution from the 384-well filter plate into a 384-well catch plate by centrifugation. The eluted nucleic acids in this condition were amenable to analysis by ESI-MS (See Figure 2). The time required for purification of samples in a single 96-well plate using a liquid handler is approximately five minutes.

Example 5: Comparison of the ZipTips™ Purification method with the Solution Capture Method

To investigate the efficacy of the solution capture method of the present invention, the ESI-MS analysis results obtained for PCR products purified with the solution capture method (Example 4) were compared with the ZipTips™ method outlined in Example 3.

Bacillus anthracis DNA was isolated and amplified by PCR using a primer pair that amplifies a section of the lef gene of *B. anthracis* ranging from residues 756-872. Shown in Figure 2 is a comparison of ESI-MS spectra for purified PCR products obtained by purification with ZipTips™ (top panel) and by the solution capture purification method of the present invention. The comparison indicates that purification by the solution capture method is equally effective as the previously validated method which employs ZipTips™. However, purification by solution capture represents a significant cost savings and is more efficient. As stated by Jiang and Hofstadler, purification of PCR products using ZipTips™ "yields an ESI-compatible sample (96-well plate) in less than 20 minutes." The solution capture method of the present invention yields an ESI-compatible sample in approximately five minutes. The retail cost of the current procedure using a pipette tip packed with anion exchange resin exemplified by ZipTip™ AX (Millipore, Bedford, MA) is approximately $1.77 per pipette tip (for each sample). The estimated cost of solution capture of PCR products is $0.10 per sample and takes into account the combination of anion exchange resin and filter plate.

Various modifications of the invention, in addition to those described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. Each reference cited in the present application is incorporated herein by reference in its entirety. U.S. provisional application Serial No. 60/470,547 filed May 13, 2003 is incorporated herein by reference in its entirety.

CLAIMS

What is claimed is:

1. A method of purifying a solution comprising a nucleic acid comprising:

mixing said solution with an anion exchange resin to yield a suspension of said anion exchange resin in said solution wherein said nucleic acids bind to said anion exchange resin;

isolating said anion exchange resin from said solution;

washing said anion exchange resin to remove one or more contaminants with one or more wash buffers while retaining bound nucleic acids;

eluting said nucleic acids from said ion exchange resin with an elution buffer.

2. The method of claim 1 further comprising analyzing said nucleic acid by mass spectrometry.

3. The method of claim 1 wherein said mixing, washing, and eluting steps are performed by a liquid handler.

4. The method of claim 1 wherein said mixing, washing, isolating, and eluting steps are performed by a robot.

5. The method of claim 1 wherein said anion exchange resin is a strong anion exchange resin.

6. The method of claim 5 wherein the functional group of said strong anion exchange resin is a quaternary amine.

7. The method of claim 1 wherein said anion exchange resin is a weak anion exchange resin.

8. The method of claim 7 wherein the functional group of said weak anion exchange resin comprises polyethyleneimine, a charged aromatic amine, diethylaminomethyl, or diethylaminoethyl.

9. The method of claim 7 wherein said weak anion exchange resin comprises one of the following commercial products: Baker polyethyleneimine resin, Baker diethylaminomethyl resin, Dionex ProPac™ WAX, Millipore polyethyleneimine, and Applied Biosystems POROS™ PI.

10. The method of claim 1 wherein said mixing is accomplished by pipetting, vortexing, sonication, or shaking.

11. The method of claim 1 wherein said isolating step is accomplished by filtration.

12. The method of claim 11 wherein said filtration is carried out in a 96-well plate, a 384-well plate, or a micro filter tube.

13. The method of claim 11 wherein said filtration is accomplished employing one of the following membranes: polytetrafluoroethylene, polyvinyldifluoroethylene, polypropylene, polyethylene, glass fiber, polycarbonate or polysulfone.

14. The method of claim 11 wherein said filtration is accomplished by vacuum, centrifugation or positive pressure displacement with fluids or gases.

15. The method of claim 1 wherein said anion exchange resin comprises an anion exchange functional group bound to a magnetic bead.

16. The method of claim 15 wherein said isolating step is accomplished by application of a magnetic field to compress said magnetic beads and aspirating said solution.

17. The method of claim 1 wherein said wash buffer comprises ammonium acetate.

18. The method of claim 1 wherein said wash buffer comprises ammonium bicarbonate.

19. The method of claim 1 wherein said wash buffer comprises methanol.

20. The method of claim 1 wherein said elution buffer comprises ammonium hydroxide.

21. The method of claim 1 wherein said elution buffer is compatible with electrospray ionization (ESI).

22. The method of claim 1 wherein said elution buffer comprises a combination of piperidine, imidazole and methanol.

23. The method of claim 1 wherein said solution of nucleic acids is the product of a nucleic acid amplification reaction.

24. The method of claim 23 wherein said amplification reaction is effected by the polymerase chain reaction, the ligase chain reaction or strand displacement amplification.

25. The method of claim 1 wherein said contaminants comprise any of the following: buffer salts, stabilizers, metal cations, proteins and deoxynucleotide triphosphates.

26. A method of purifying a solution comprising a nucleic acid for subsequent analysis by electrospray mass spectrometry comprising:

adding a weak anion exchange resin to said solution and mixing to yield a suspension of said weak anion exchange resin in said solution wherein said nucleic acids bind to said weak anion exchange resin;

isolating said weak anion exchange resin from said solution;

washing said weak anion exchange resin to remove contaminants with one or more wash buffers while retaining bound nucleic acids; and

eluting said nucleic acids from said weak ion exchange resin with an elution buffer.

27. The method of claim 26 further comprising analyzing said nucleic acid by mass spectrometry.

28. The method of claim 26 wherein the functional group of said weak anion exchange resin comprises a polyethyleneimine, charged aromatic amine, diethylaminomethyl, or diethylaminoethyl.

29. The method of claim 26 wherein said weak anion exchange resin comprises one of the following commercial products: Baker polyethyleneimine resin, Baker diethylaminomethyl resin, Dionex ProPac WAX, Millipore polyethyleneimine, and Applied Biosystems POROS PI.

30. A method of purifying a solution of nucleic acids for subsequent analysis by electrospray mass spectrometry comprising:

adding ProPac™ WAX weak anion exchange resin to said solution and mixing to yield a suspension of said weak anion exchange resin in said solution wherein said nucleic acids bind to said weak anion exchange resin;

isolating said weak anion exchange resin from said solution;

washing said weak anion exchange resin to remove contaminants with one or more wash buffers while retaining bound nucleic acids; and

eluting said nucleic acids from said weak ion exchange resin with an elution buffer comprising piperidine, imidazole and methanol.

31. The method of claim 30 further comprising analyzing said nucleic acid by mass spectrometry.

32. A kit for purification of nucleic acids comprising:

a resin source plate containing anion exchange resin;

a sample mixing plate;

a filter plate or a microtube rack comprising a plurality of micro filter tubes;

one or more resin wash solutions; and

an ESI-MS-compatible elution buffer.

33. The kit of claim 32 wherein said filter plate is a 96-well plate or a 384-well plate.

34. The kit of claim 32 wherein said anion exchange resin comprises a weak anion exchange functional group.

35. The kit of claim 32 wherein said anion exchange resin comprises ProPac™ WAX.

36. The kit of claim 32 wherein said anion exchange resin comprises a weak anion exchange functional group bound to a magnetic bead.

37. The kit of claim 32 wherein said weak anion exchange resin is ProPac™ WAX.

38. The kit of claim 32 wherein said one or more wash solutions comprises about 50 mM to about 200 mM ammonium acetate or about 50 mM to about 200 mM ammonium bicarbonate.

39. The kit of claim 32 wherein said at least one anion exchange wash buffer comprises about 20% to about 50% methanol.

40. The kit of claim 32 wherein said ESI-MS compatible elution buffer comprises about 2% to about 6% ammonium hydroxide.

41. The kit of claim 32 wherein said ESI-MS compatible elution buffer comprises about 10 mM to about 50 mM imidazole, about 10 mM to about 50 mM piperidine and about 20% to about 50% methanol.

42. The kit of claim 32 wherein said ESI-MS compatible elution buffer comprises about 10 mM to about 50 mM imidazole, about 10 mM to about 50 mM piperidine and about 20% to about 50% methanol.

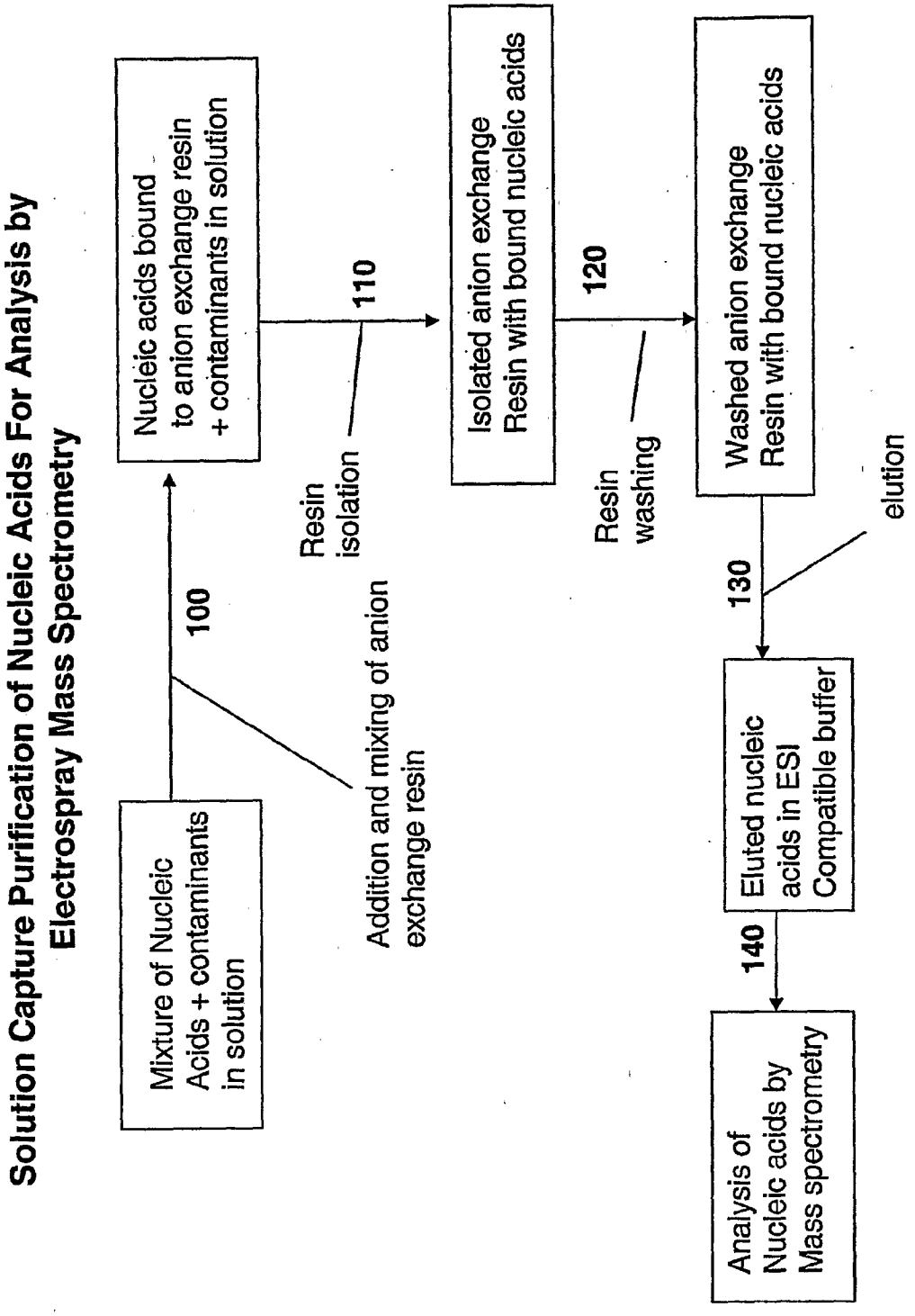
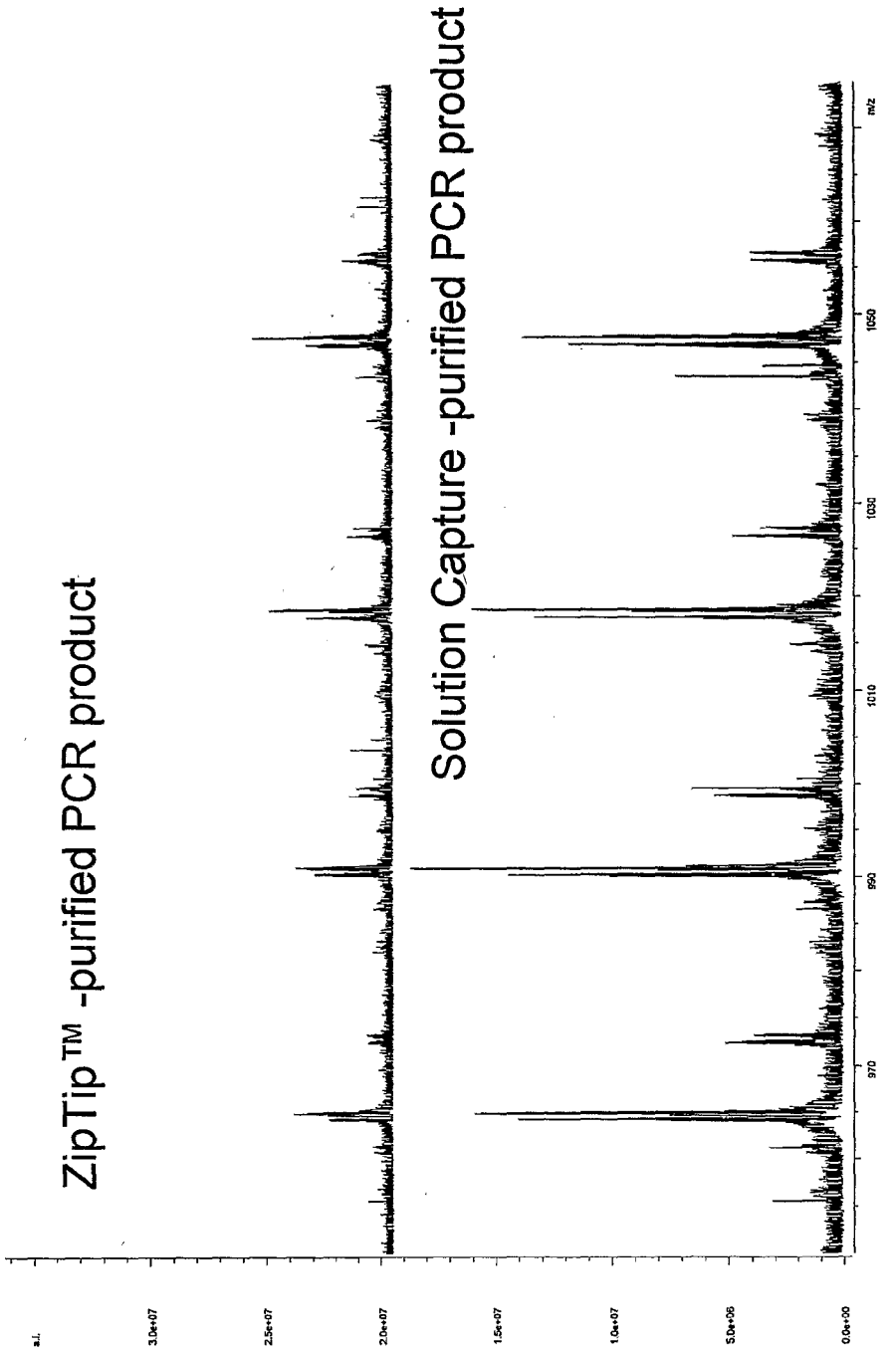

Solution Capture Purification of Nucleic Acids For Analysis by Electrospray Mass Spectrometry
Mixture of Nucleic Acids + contaminants in solution
100
Addition and mixing of anion exchange resin
Nucleic acids bound to anion exchange resin + contaminants in solution
Resin isolation
110
Isolated anion exchange Resin with bound nucleic acids
120
Resin washing
Washed anion exchange Resin with bound nucleic acids
130
elution
Eluted nucleic acids in ESI Compatible buffer
140
Analysis of Nucleic acids by Mass spectrometry

Figure 1

Figure 2


ZipTip™ -purified PCR product
Solution Capture -purified PCR product
a.u.
3.0e+07
2.5e+07
2.0e+07
1.5e+07
1.0e+07
5.0e+06
0.0e+00
970
990
1010
1030
1050
m/z